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EVIDENCE FOR THE UTILIZATION OF GALACTOSE BY NORMAL AND TRANSFORMED PLANT CELLS IN CULTURE[1]

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Abstract

Sucrose may be substituted with galactose in Murashige-Skoog medium for the growth of *Vinca rosea* callus cells, and *V. rosea* cells were able to utilize galactose as efficiently as sucrose. However, the growth of crown gall tumor cells (transformed by *Agrobacterium tumefaciens*) was 4-fold greater than that of normal cells (after 16 days at 25°C).

Since both normal and crown gall tumor cells grow on galactose, a point is raised whether genetic transformations in higher plants are actually due to: (a) the expression of bacterial genes in plant cells; (b) fortuitous cloning brought about by positive selection procedures; or (c) expression of an adaptive gene in plants analogous to newly generated secondary β-galactosidase in bacteria.

Introduction

Plant cells are normally grown in culture media containing glucose, fructose, or sucrose with the latter carbon source being the most commonly used (Street, 1966; Yeoman, 1973). Other sugars, particularly galactose, have not been used for culturing plant cells because they are not metabolized or are extremely toxic to plants (Stenlid, 1959; Malca *et al.*, 1967; Faludi *et al.*, 1963; Hoffmann *et al.*, 1971; Göring *et al.*, 1968; Roberts *et al.*, 1971; Hoffmannowa, 1964). Recently, plant tissue cultures have been reported to be apparently genetically transformed by bacterial DNA and bacterial DNA mediated through phages (Doy *et al.*, 1973; Johnson *et al.*, 1973; Hess, 1972). These transformed cells are able to utilize either galactose or lactose. The utilization of apparently unusable or toxic sugars such as galactose or lactose through genetic transformation of plant cells with foreign DNA was of particular interest to us

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since recent reports have suggested the presence of *Agrobacterium tumefaciens* DNA in cultured crown gall tumor cells (Kado, 1976).

We wondered, therefore, whether bacterial genes for utilizing these sugars are present in crown gall tumor cells and not in normal cells. If so, only tumor cells should be able to grow on media containing galactose. We found, however, that both normal and tumor cells of *V. rosea* grew as well on media containing galactose as on media containing sucrose. In surveying the earlier works on plant tissue culture, we found a few exceptions in which tissue culture explants from plants such as cucumber (*Cucumis sativus*), redwood (*Sequoia sempervirens*), sugar maple (*Acer sacchrum*), and carrots (*Daucus carota*) were able to grow to a limited extent on media containing lactose or galactose (Göring *et al.*, 1968; Ball, 1955; Mathes *et al.*, 1971; Gautheret, 1945).

Materials and Methods

Vinca rosea cell culture clones were kindly provided originally from Dr. Armin C. Braun, Rockefeller University, New York, and from Dr. Robert Manasse, Boyce Thompson Institute, New York. Uncloned stem cultures were also prepared in our laboratory. All callus cultures were grown on synthetic and defined agar medium of Murashige and Skoog (1962) substituting sucrose with the experimental carbon source. These cultures were systematically checked for bacterial contamination in broth medium 523 (Kado *et al.*, 1972). No bacterial contamination occurred at any point in the experiments. All cultures were grown in the dark at 25°C unless specified otherwise. Crystalline D-galactose and D-galactose-1-^{14}C were purchased from Sigma Chemical Company, St. Louis, Missouri and Amersham/Searle Corporation, Arlington Heights, Illinois, respectively. Both lots of galactose were checked for purity by paper chromatography. Three chromatographic solvents were used: (1) *n*-butanol : acetic acid : water (4:1:5, v/v/v); (2) isopropanol : pyridine : water : acetic acid (8:8:4:1, v/v/v/v); and (3) *n*-butanol : ethanol : water, (10:1:2, v/v/v). Galactose was extracted from agar medium before and after autoclaving. No deterioration or alterations of extracted galactose were detected.

Growth studies were conducted by transferring approximately 1 g fresh weight of *V. rosea* tissues to medium containing various concentrations of galactose. Unless otherwise specified, all mother cultures of *V. rosea* had been maintained for at least 6 transfers on media containing the experimental sugar to eliminate the possibility of residual sucrose that could remain in the initial transfer. For uptake and utilization studies, preweighed callus tissues were frozen and homogenized in a mortar with 10% trichloroacetic acid. The precipitates were washed with 95% ethanol and the radioactivity was measured as before (Kado *et al.*, 1972). This approach is resonable because it has been

shown that definitive growth studies can be made by determining the dry or wet weights of tissues since such weights are proportional to each other (Hildebrandt and Riker, 1949). We measured growth by taking fresh weight measurements and expressed the amount of growth as the percent increase in weight from time zero.

Results and Discussion

Growth of both normal and tumor tissues on galactose containing medium is demonstrated in Fig. 1. The growth of tumor cells after 16 days was 4-fold greater than that of normal cell lines. The rate of increase in wet weight of tumor cells was about 30% per day compared to 7% per day for normal cells. These data suggest that galactose was metabolized as efficiently as sucrose, also no toxic effects of galactose were seen (macroscopically or microscopically). Furthermore, the concentration of galactose was not limiting within the ranges used (Fig. 2). This showes again that galactose can be metabolized and is responsible for growth. Fig. 3 shows that ^{14}C-

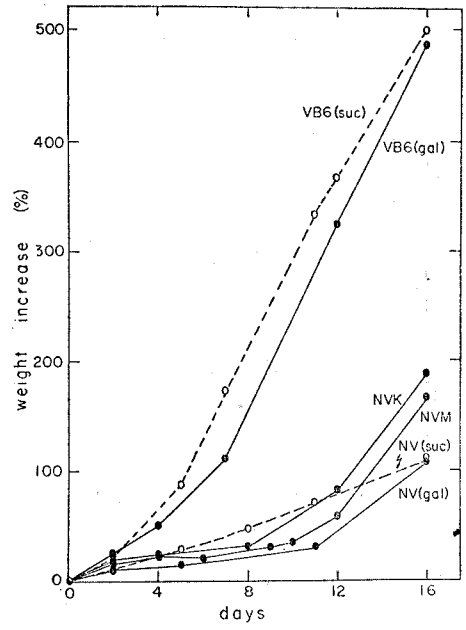

Fig. 1. Growth of normal and crown gall tumor tissues of *Vinca rosea* at 25°C as a function of time (days) on synthetic-1% agar medium (Murashige and Skoog, 1962) containing either 1% sucrose (suc) (dash lines) or 1% galactose (gal) (solid lines). Each point represents the average of 6 determinations. VB_6=crown gall tumor tissue; NVM=normal tissue from Robert Manasse, Boyce Thompson Institute, Yonkers, New York; NV=normal tissue from Armin Braun, Rockefeller University, New York; NVK=normal tissue (our line at Davis).

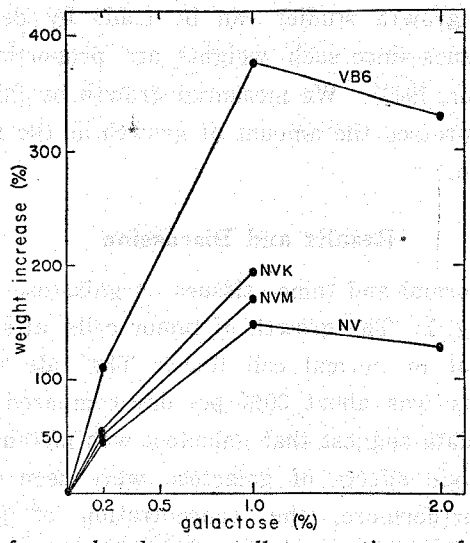

Fig. 2. Growth of normal and crown gall tumor tissues of *Vinca rosea* as a function of D-galactose concentration. Each point represents the average of 4 determinations taken after 20 days growth at 25°C. Tissue designations are the same as in Fig. 1.

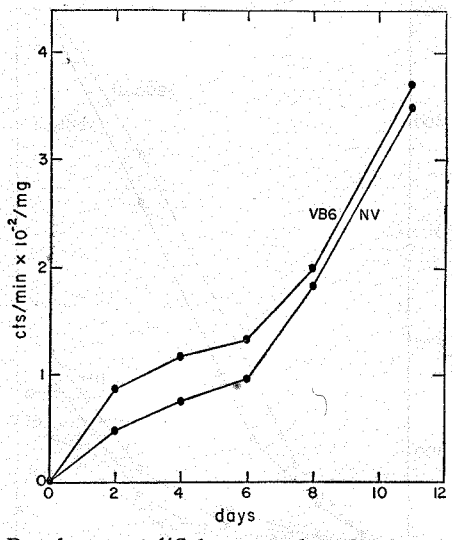

Fig. 3. Uptake of D-galactose-1-^{14}C by normal and crown gall tumor tissues of *Vinca rosea* as a function of time (days). Tissue samples were allowed to grow at 25°C on synthetic 1% agar medium (Murashige and Skoog, 1962) (without sucrose) containing 130 mμ Ci/ml of D-galactose-1-^{14}C (59 m Ci/m mol) and 0.5% galactose. At various times, tissue samples were removed from the marginal meristematic regions as per Ball (1955), weighed, and washed with 30 ml of cold 0.5% galactose medium three times. The tissue samples were dried, reweighed, and radioactivity was measured in a liquid scintillation spectrometer (Beckman/LS233). Each point represents the average of three determinations.

galactose uptake was about the same in both normal and tumor meristematic cells (184 and 200 cpm/mg of normal and tumor meristematic tissues, respectively after 8 days) although total callus tissue of tumors has a higher specific radioactivity than total callus tissue of normal cells (37 cpm/mg and 18 cpm/mg, respectively) due to its faster growth rate.

Our demonstration of the efficient utilization of galactose by normal plant cells for growth, raises the point of whether genetic transformations recently reported with tomato and sycamore cultures are due to: (a) the expression of bacterial genes in plant cells, (b) fortuitous tissue cloning brought about by positive selection procedures, or (c) expression of an adaptive gene in plants analogous to newly generated enzymes such as a secondary β-galactosidase in bacteria (Campbell *et al.*, 1973). In the case of fortuitous cloning, the example of crown gall can be used. Crown gall callus cultures can grow efficiently on lactose (Hildebrandt and Riker, 1949) and such cultures were shown here to grow faster than normal cells on galactose. Galactosidases have been recently shown to occur in plants (Agrawal and Bahl, 1968). Tumor cells are known to be more permeable than normal cells (Wood and Braun, 1965). Thus the somewhat higher levels of permeability of tumor cells to galactose may be the reason for elevated growth rates and utilization of an uncommon carbon source. Likewise, an adaptive gene for galactose utilization could give the same result as transformed cells. Hence the possibility clearly exists that some of the genetic transformation experiments reported in various laboratories using cell cultures different from each other may be due to clonal selection of cells with either higher permeability or adaptive genes since such experiments entail positive selection procedures.

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Literature Cited

AGRAWAL, K. M. L. and O. P. BAHL. 1968. Glycosidases of *Phaseolus vulgris*. II. Isolation and general praperties. J. Biol. Chem. **243**: 103-111.

BALL, E. 1955. Studies of the nutrition of the callus culture of *Sequoia sempervirens*. Annee Biol. **31**: 81-105.

CAMPBELL, J. H., J. A. LENGYEL, and J. LANGRIDGE. 1973. Evolution of a second gene for β-galactosidase in *Escherichia coli*. Proc. Nat. Acad. Sci., U. S. A. **70**: 1841-1845.

DOY, C. H., P. M. GRESSHOFF, and B. G. ROLFE. 1973. Biological and molecular evidence for the transgenesis of genes from bacteria to plant cells. Proc. Nat. Acad. Sci., U. S. A. **70**: 723-727.

FALUDI, B., A. F. DANIEL, I. GYURJAN, and S. ANDA. 1963. Sugar antagonisms in plant

tumor cells induced by 2,4-dichlorophenoxyacetic acid. Acta Biol. Acad. Sci. Hungary. **14**: 183-190.

GAUTHERET, R. J. 1945. Une voie nouvelle en biologie vegetale: La culture des tissus: Gallimard, Paris. p. 128.

GÖRING, V. H., E. RECKIN, and M. V. R. KAISER. 1968. Einfluss der D-galaktose anf den kohlenhydratstoffwechsel pflanzlicher gewebe. Physiol. Biochem. **159**: 82-103.

HESS, D. 1972. Transformations in higher organisms. Naturwissenschaften **59**: 348-355.

HILDEBRANDT, A. C. and A. J. RIKER. 1949. The influence of various carbon compounds on the growth of marigold, paris-daisy, periwinkle, sunflower and tobacco tissue *in vitro*. Amer. J. Bot. **36**: 74-85.

HOFFMANN, F., U. KULL, and K. JEREMIAS. 1971. Markierung von kohlenhydraten in blähern nach fütterung von ^{14}C-galaktose. Z. Pflanzenphysiol. **64**: 223-231.

HOFFMANNOWA, A. 1964. Some aspects of sugar nutrition of excised embryos of *Lupinus luteus* L. and *Brassica oleracea* L. Acta. Soc. Botan. Polon. **33**: 193-210.

JOHNSON, C. B., D. GRIERSON, and H. SMITH. 1973. Expression of λplac 5 DNA in cultured cells of a higher plant. Nature New Biol. **244**: 105-107.

KADO, C. I., 1976. The tumor-inducing substance of *Agrobacterium tumefaciens*. Ann. Rev. Phytopath. **14**: 265-308.

KADO, C. I., M. G. HESKETT, and R. A. LANGLEY. 1972. Studies of *Agrobactarium tumefaciens*. Characterization of strains 1D135 and B6, and analysis of the bacterial chromosome transfer RNA and ribosomes for tumor inducing ability. Physiol. Pl. Pathol. **2**: 47-57.

MALCA, I., R. M. ENDO, and M. R. LONG. 1967. Mechanism of glucose counteraction of inhibition of root elongation by galactose, mannose, and glucosamine. Phytopath. **57**: 272-281.

MATHES, M. C., M. MORSELLI, and J. W. MARVIN. 1971. The in vitro growth and metabolism of *Acer saccharum* tissue. Can. J. Bot. **49**: 495-500.

MURASHIGE, T., and F. SKOOG. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. **15**: 473-497.

ROBERTS, R. M., A. HEISHMAN, and C. WICKLIN. 1971. Growth inhibition and metabolite pool levels in plant tissues fed D-galactosamine and D-galactose. Plant Physiol. **48**: 36-42.

SCHILPEROORT, R. A., N. J. VAN SITTERT, and J. SCHELL. 1973. The presence of both phage PS8 and *A. tumefaciens* A6 DNA base sequences in A6 induced sterile crown-gall tissue cultured *in vitro*. Eur. J. Biochem. **33**: 1-7.

STENLID, G. 1959. Species differences between plant roots in the reaction to inhibitory sugars. Physiol. Plant. **12**: 218-235.

STREET, H. E. 1966. The nutrition and metabolism of plant tissue and organ cultures. *In* E. N. WILMER (ed.), The biology of cells and tissues in culture, Vol. 3, Academic Press, N. Y.

WOOD, H. N. and A. C. BRAUN. 1965. Studies on the net uptake of solutes by normal and crown-gall tumor cells. Proc. Nat. Acad. Sci. (U. S. A.) **54**: 1532-1538.

YEOMAN, M. M. 1973. Tissue (callus) cultures—Techniques. *In* H. E. STREET (ed.), Plant tissues and cell culture, Botanical Monographs Vol. 11, Univ. of Calif. Press, Berkeley and Los Angeles, California.

長春花的正常和癌腫細胞能利用半乳糖為碳素源的研究

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植物組織培養細胞，一般而言大多可利用葡萄糖，果糖或蔗糖爲碳素源，而半乳糖常對植物組織培養細胞有毒性作用。這篇報告首次指出長春花的正常組織培養細胞能利用半乳糖爲碳素源。癌腫病菌所轉化成的癌腫組織培養細胞在半乳糖爲碳素源的 Murashige 和 Skoog培養基上生長速率較正常組織培養細胞快四倍左右。因此我們懷疑這種癌腫細胞的快速生長是由於其細胞膜的透析性比正常細胞膜大，或是癌腫細胞內有細菌（*Agrobacterium tumefaciens*）的半乳糖分解酵素的作用，或是由於癌腫細胞體內有新的能適應利用此半乳糖的新基因出現。由於最近有不少報告指出植物細胞能利用細菌的半乳糖分解酵素的基因而使原來不生長在半乳糖培養基上的植物細胞變爲能生長。我們覺得這方面的研究，有待更進深入的探討，尤其是去證明轉化爲能利用半乳糖的植物細胞體內的半乳糖分解酵素和細菌體內者的一樣。